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United States Patent [19]
Moses

[11] **Patent Number:** **5,474,920**
[45] **Date of Patent:** **Dec. 12, 1995**

[54] **MODIFIED THERMO-RESISTANT DNA
POLYMERASES**

[75] Inventor: **Robb E. Moses**, Portland, Oreg.

[73] Assignee: **State of Oregon, Acting by and
Through the Oregon State Board of
Higher Education on Behalf of the
Oregon Health Sciences University,**
Portland, Oreg.

[21] Appl. No.: **156,020**

[22] Filed: **Nov. 23, 1993**

[51] Int. Cl.⁶ **C12N 9/12**

[52] U.S. Cl. **435/194; 435/252.3; 536/23.2;
935/10; 935/14**

[58] Field of Search **435/194, 252.3;
536/23.2; 935/10, 14**

[56] **References Cited**

U.S. PATENT DOCUMENTS

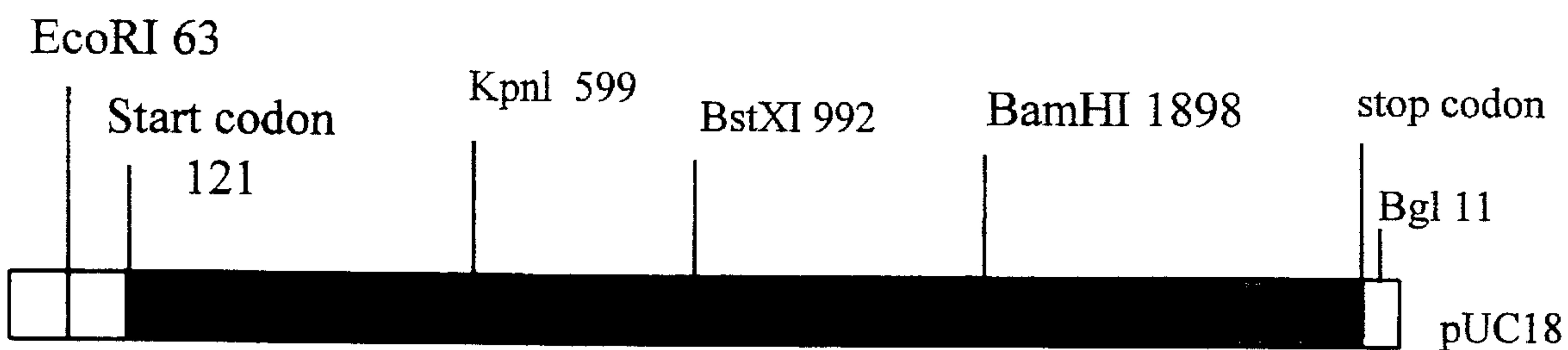
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Long



Restriction map of gene for Taq DNA polymerase

FIG. 1

Scheme for Zone Mutagenesis

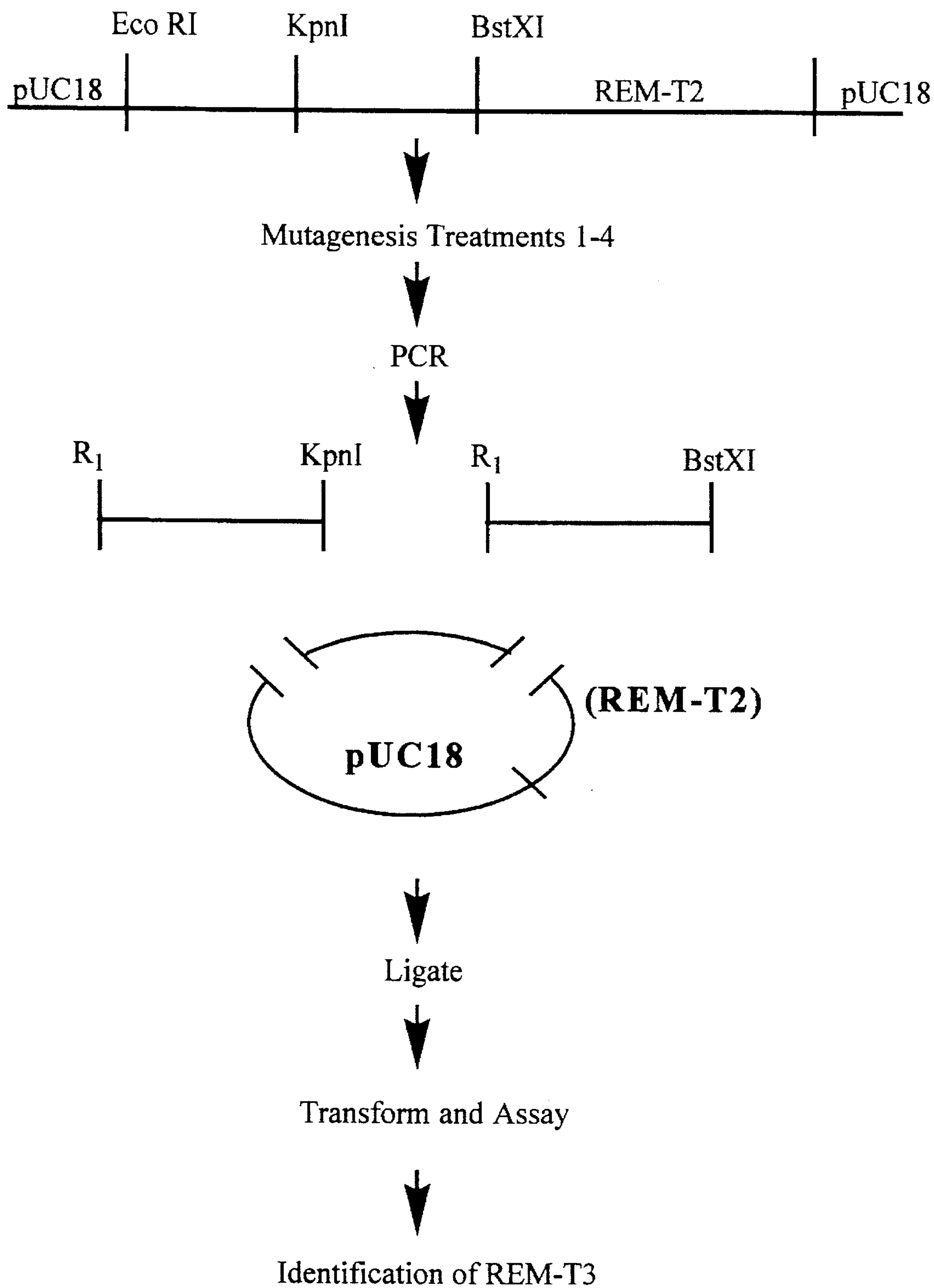


FIG. 2

Sequencing Primers for pLSM5

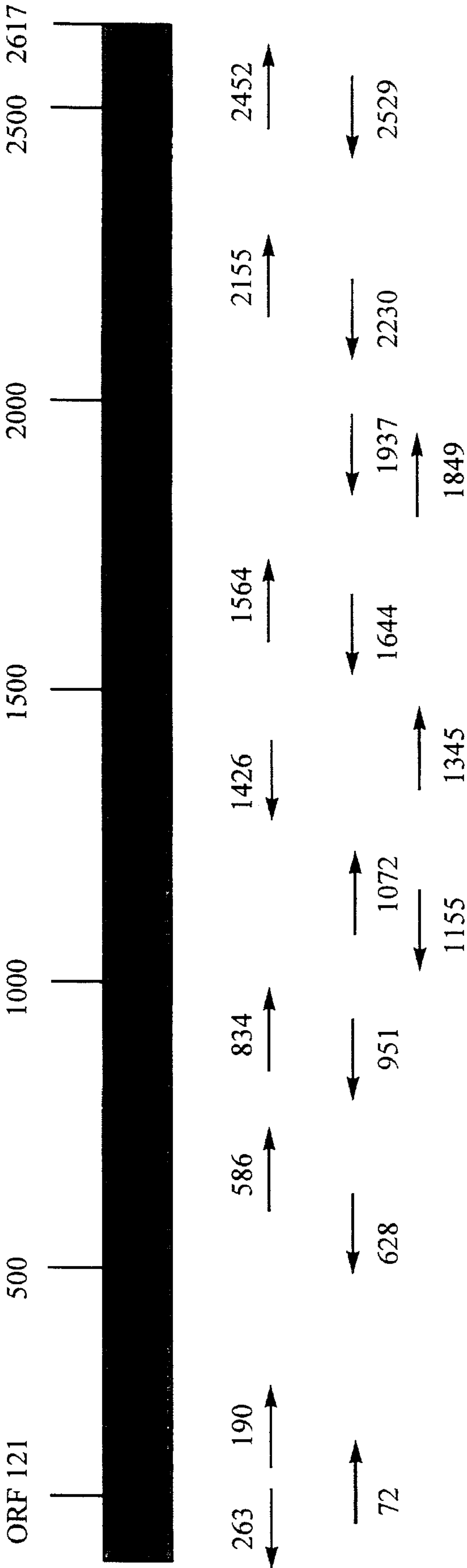


FIG. 3

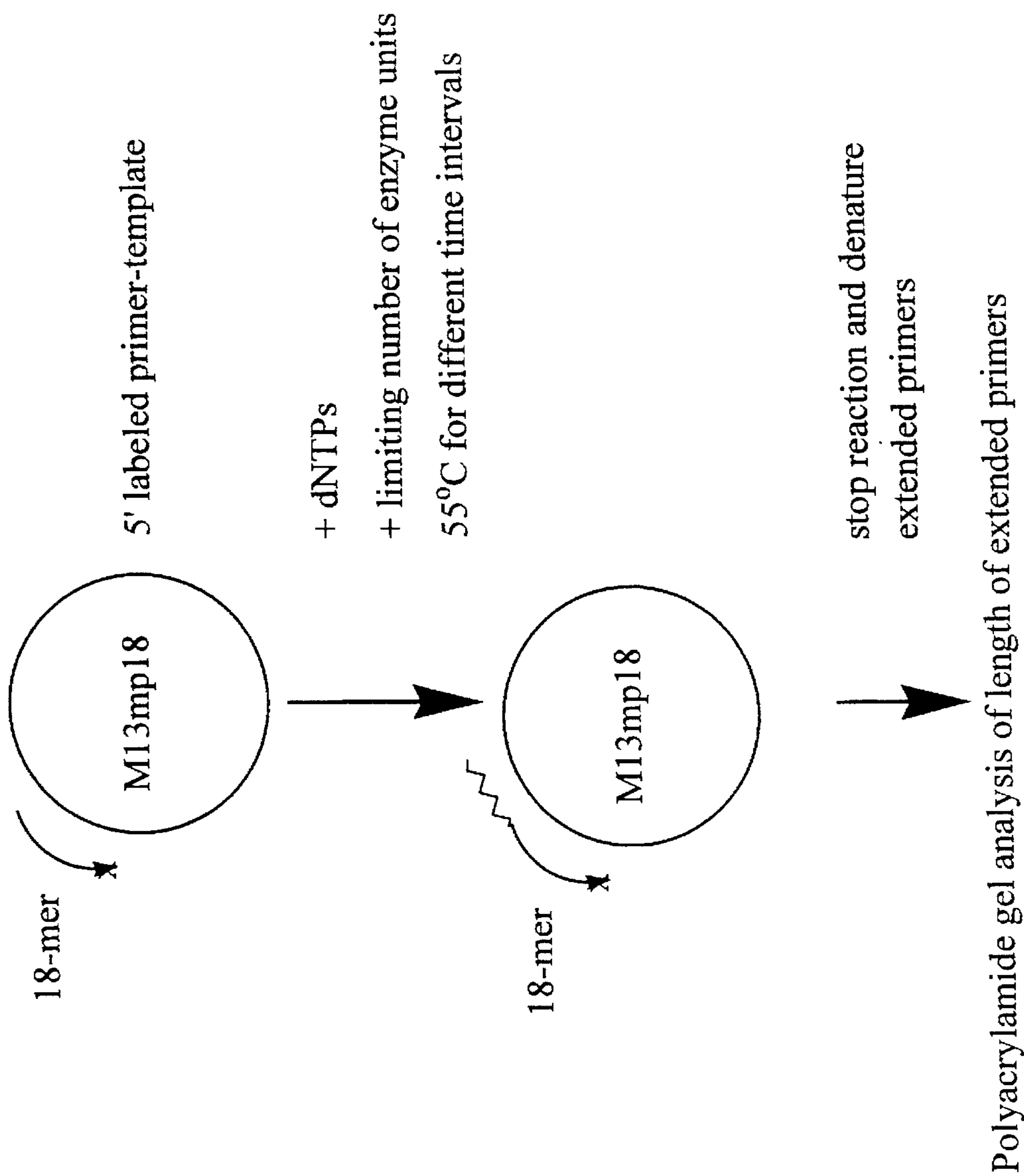


FIG. 4

PCR Analysis of Processivity

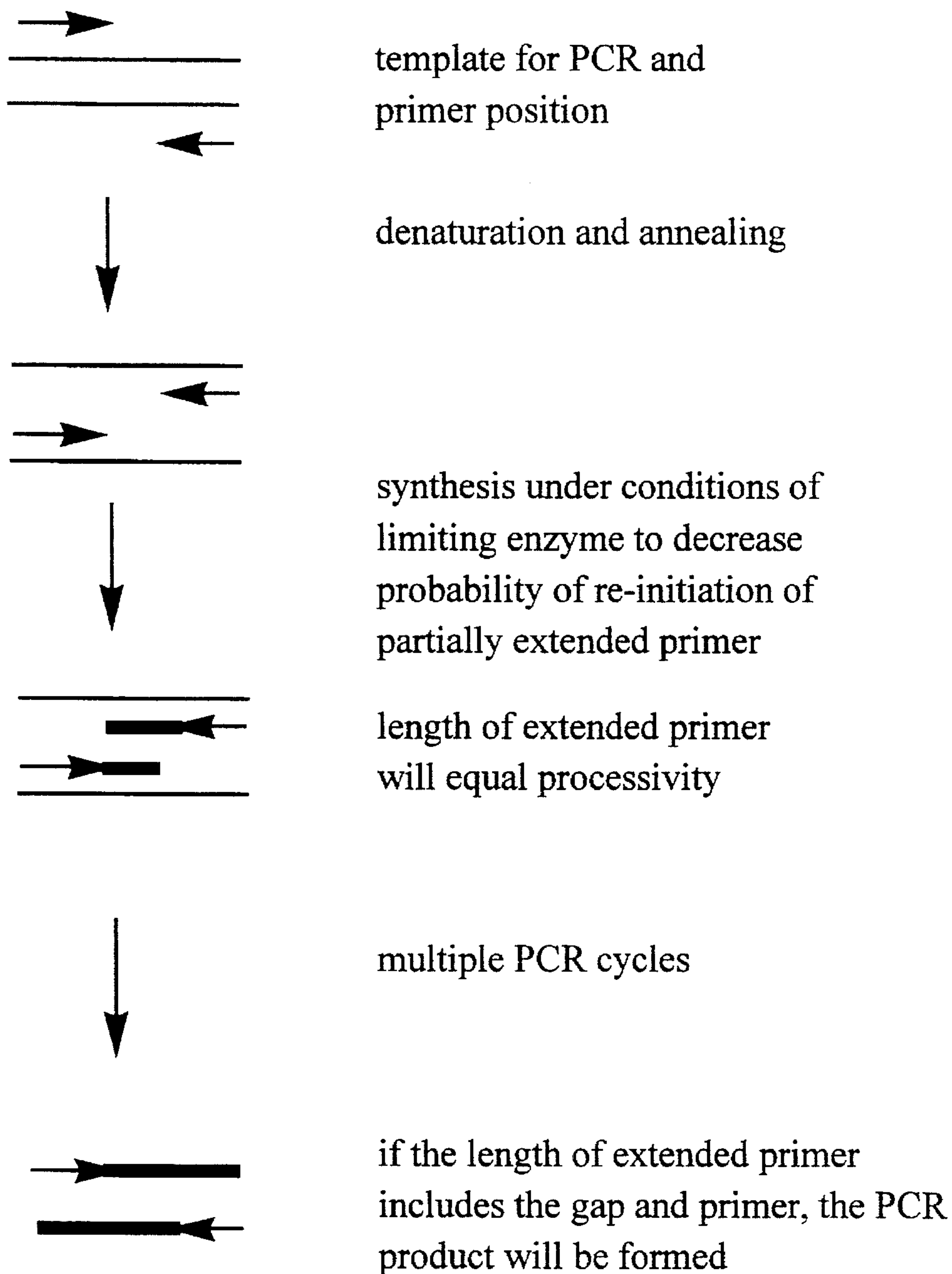


FIG. 7

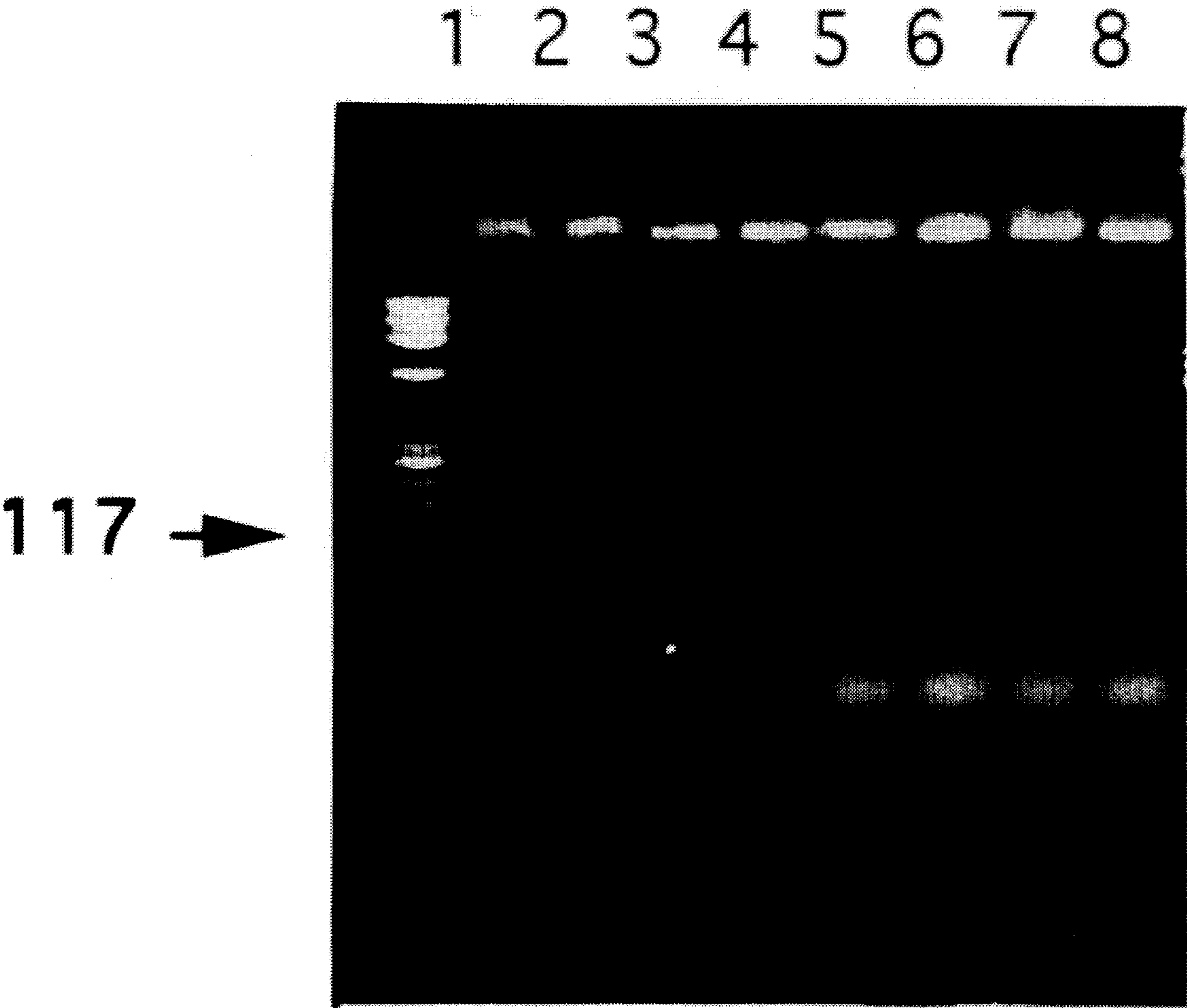


FIG. 8

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CTC Leu	TCC Ser	TGG Trp	GAC Asp	CTG Leu 245	GCC Ala	AAG Lys	GTG Val	CGC Arg	ACC Thr 250	GAC Asp	CTG Leu	CCC Pro	CTG Leu	GAG Glu 255	GTG Val	888
GAC Asp	TTC Phe	GCC Ala	AAA Lys 260	AGG Arg	CGG Arg	GAG Glu	CCC Pro	GAC Asp 265	CGG Arg	GAG Glu	AGG Arg	CTT Leu	AGG Arg 270	GCC Ala	TTT Phe	936
CTG Leu	GAG Glu	AGG Arg 275	CTT Leu	GAG Glu	TTT Phe	GGC Gly	AGC Ser 280	CTC Leu	CTC Leu	CAC His						

-continued

CAC His	ACC Thr	CGC Arg	TTC Phe	AAC Asn 565	CAG Gln	ACG Thr	GCC Ala	ACG Thr	GCC Ala 570	ACG Thr	GGC Gly	AGG Arg	CTA Leu	AGT Ser 575	AGC Ser	1848
TCC Ser	GAT Asp	CCC Pro	AAC Asn 580	CTC Leu	CAG Gln	AAC Asn	ATC Ile	CCC Pro 585	GTC Val	CGC Arg	ACC Thr	CCG Pro	CTT Leu 590	GGG Gly	CAG Gln	1896
AGG Arg	ATC Ile	CGC Arg 595	CGG Arg	GCC Ala	TTC Phe	ATC Ile	GCC Al									

-continued

(i i) MOLECULE TYPE: protein															
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:															
Met	Arg	Gly	Met	Leu	Pro	Leu	Phe	Glu	Pro	Lys	Gly	Arg	Val	Leu	Leu
1				5					10					15	
Val	Asp	Gly	His	His	Leu	Ala									

-continued

Glu	Ala	Gly	Glu	Arg 405	Ala	Ala	Leu	Ser	Glu 410	Arg	Leu	Phe	Ala	Asn 415	Leu
Trp	Gly	Arg	Leu 420	Glu	Gly	Glu	Glu	Arg 425	Leu	Leu	Trp	Leu	Tyr 430	Arg	Glu
Val	Glu	Arg 435	Pro	Leu	Ser	Ala	Val 440	Leu	Ala	His	Met	Glu 445	Ala	Thr	Gly
Val	Arg 450	Leu	Asp	Val	Ala	Tyr 455	Leu	Arg	Ala	Leu	Ser 460	Leu	Glu	Val	Ala
Glu 465	Glu	Ile	Ala	Arg	Leu 470	Glu									

-continued

8 2 0	8 2 5	8 3 0
(2) INFORMATION FOR SEQ ID NO:3:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH: 2626 base pairs		
(B) TYPE: nucleic acid		
(C) STRANDEDNESS: single		
(D) TOPOLOGY: linear		
(i i) MOLECULE TYPE: DNA (genomic)		
(i i i) HYPOTHETICAL: NO		
(i v) ANTI-SENSE: NO		
(v i) ORIGINAL SOURCE:		
(A) ORGANISM: Thermus aquaticus		

-continued

ATG Met	TCG Ser	GCC Ala 675	CAC His	CGC Arg	CTC Leu	TCC Ser	CAG Gln 680	GAG Glu	CTA Leu	GCC Ala	ATC Ile	CCT Pro 685	TAC Tyr	GAG Glu	GAG Glu	2184
GCC Ala 690	CAG Gln	GCC Ala	TTC Phe	ATT Ile	GAG Glu	CGC Arg 695	TAC Tyr	TTT Phe	CAG Gln	AGC Ser	TTC Phe 700	CCC Pro	AAG Lys	GTG Val	CGG Arg	2232
GCC Ala 705	TGG Trp	ATT Ile	GAG Glu	AAG Lys	ACC Thr 710	CTG Leu	GAG Glu	GAG Glu	GGC							

-continued

Ala	Glu	Lys	Glu	Gly	Tyr	Glu	Val	Arg	Ile	Leu	Thr	Ala	Asp	Lys	Asp
	130					135					140				
Leu	Tyr	Gln	Leu	Leu	Ser	Asp	Arg	Ile	His	Val	Leu	His	Pro	Glu	Gly
145					150					155					160
Tyr	Leu	Ile	Thr	Pro	Ala	Trp	Leu	Trp	Glu	Lys	Tyr	Gly	Leu	Arg	Pro
				165											

-continued

545					550					555					560				
His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser				
				565					570					575					
Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly	Gln				

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(A) NAME/KEY: mutation	
(B) LOCATION: replace(934, "a")	
(D) OTHER INFORMATION: /note="This mutation results in a nucleotide alteration at position 934 of the native Taq DNA polymerase nucleotide sequence of T to A. This results in an amino acid change of Phe to Ile."	
(i x) FEATURE:	
(A) NAME/KEY: mutation	
(B) LOCATION: replace(962, "c")	
(D) OTHER INFORMATION: /note="This mutation results in a nucleotide alteration at position 962 of the native Taq DNA polymerase nucleotide sequence of T to C. This results in an amino acid change of Leu to Pro."	
(i x) FEATURE:	
(A) NAME/KEY: mutation	
(B) LOCATION: replace(2535, "a")	
(D) OTHER INFORMATION: /note="This mutation results in a nucleotide alteration at position 2535 of the native Taq DNA polymerase nucleotide sequence of G to A. This mutation is conservative."	

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GAG Glu	ACC Thr	CTC Leu	TTC Phe	GGC Gly 725	CGC Arg	CGC Arg	CGC Arg	TAC Tyr	GTG Val 730	CCA Pro	GAC Asp	CTA Leu	GAG Glu	GCC Ala 735	CGG Arg	2328
GTG Val	AAG Lys	AGC Ser	GTG Val 740	CGG Arg	GAG Glu	GCG Ala	GCC Ala	GAG Glu 745	CGC Arg	ATG Met	GCC Ala	TTC Phe	AAC Asn 750	ATG Met	CCC Pro	2376
GTC Val	CAG Gln	GGC Gly 755	ACC Thr	GCC Ala	GCC Ala	GAC Asp	CTC Leu 760									

-continued

195						200						205					
Glu	Glu	Trp	Gly	Ser	Leu	Glu	Ala	Leu	Leu	Lys	Asn	Leu	Asp	Arg	Leu		
	210					215					220						
Lys	Pro	Ala	Ile	Arg	Glu	Lys	Ile	Leu	Ala	His	Met	Asp	Asp	Leu	Lys		
225																	

-continued

Asp 625	Glu	Asn	Leu	Ile	Arg 630	Val	Phe	Gln	Glu	Gly 635	Arg	Asp	Ile	His	Thr 640
Glu	Thr	Ala	Ser	Trp 645	Met	Phe	Gly	Val	Pro 650	Arg	Glu	Ala	Val	Asp 655	Pro
Leu	Met	Arg	Arg 660	Ala	Ala	Lys	Thr	Ile 665	Asn	Phe	Gly	Val	Leu 670	Tyr	Gly
Met	Ser	Ala 675	His	Arg	Leu	Ser	Gln 680	Glu	Leu	Ala	Ile	Pro 685	Tyr	Glu	Glu
Ala															

-continued

results in an amino acid change of Leu to Pro.”

(i x) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(2535, “a”)
 (D) OTHER INFORMATION: /note=“This mutation results in a
 nucleotide alteration at position 2535 of the native
 Taq DNA polymerase nucleotide sequence of G to A. This
 mutation is conservative.”

(i x) FEATURE:
 (A) NAME/KEY: mutation
 (B) LOCATION: replace(341, “a”)
 (D) OTHER INFORMATION: /note=“This mutation results in a
 nucleotide alteration at position 341 of the native Taq
 DNA polymerase nucleotide sequence of G to A. This
 mutation results in an amino acid change of Arg to His.”

(i x) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 121..2619

(i x) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) LOCATION: 121..2616

-continued

(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(i i) MOLECULE TYPE: cDNA	
(i i i) HYPOTHETICAL: NO	
(i x) FEATURE: (A) NAME/KEY: - (B) LOCATION: 1..18 (D) OTHER INFORMATION: /note="PCR reverse primer used for PUC18"	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:	
CAGGAAACAGCTATGACC	18
(2) INFORMATION FOR SEQ ID NO:12:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(i i) MOLECULE TYPE: cDNA	
(	

